iGEM 2016: Pittsburgh

Week 10 Lab Notebook

Monday, July 25

Lab meeting

Phosphorylate PT3-RBS and PT3 (Maddie/Maya)

Resuspend to 100uM with water

Phosphorylation

Made annealing buffer (Maya)

Liquid cultures of Collins switches (Claire)

Fundraising emails (Claire)

Tuesday, July 26

Miniprep liquid cultures (Claire)

Concentrations: D 1: 48.3 G 1: 72.5
D 2: 60.4 G 2: 63.1

Cell-free dilution time course with Collins plasmids (Claire)

D:	Solution A	2 uL		22 uL
	Solution B	1.5 uL		16.5 uL
	RNAse inhibitor	0.25 uL	x 11 =	2.75 uL
	Substrate	0.25 uL		2.75 uL
	Switch:	0.82 uL at 60.4 ng/uL		9.02 uL
	Trigger:	0.18 uL at 5 uM	x 8 =	1.44 uL
G:	Solution A	2 uL		20 uL
	Solution B	1.5 uL		15 uL
	RNAse inhibitor	0.25 uL	x 10 =	2.5 uL
	Substrate	0.25 uL		2.5 uL
	Switch:	0.59 uL at 84.1 ng/uL		5.9 uL
	Trigger:	0.36 uL at 2.5 uM	x 8 =	2.52 uL
	Water	0.05 uL	x 7 =	0.35 uL

Master mix:

Solution A	42 uL	
Solution B	31.5 uL	
RNAse inhibitor	5.25 uL	
Substrate	5.25 uL	

Separate out 40 uL for G

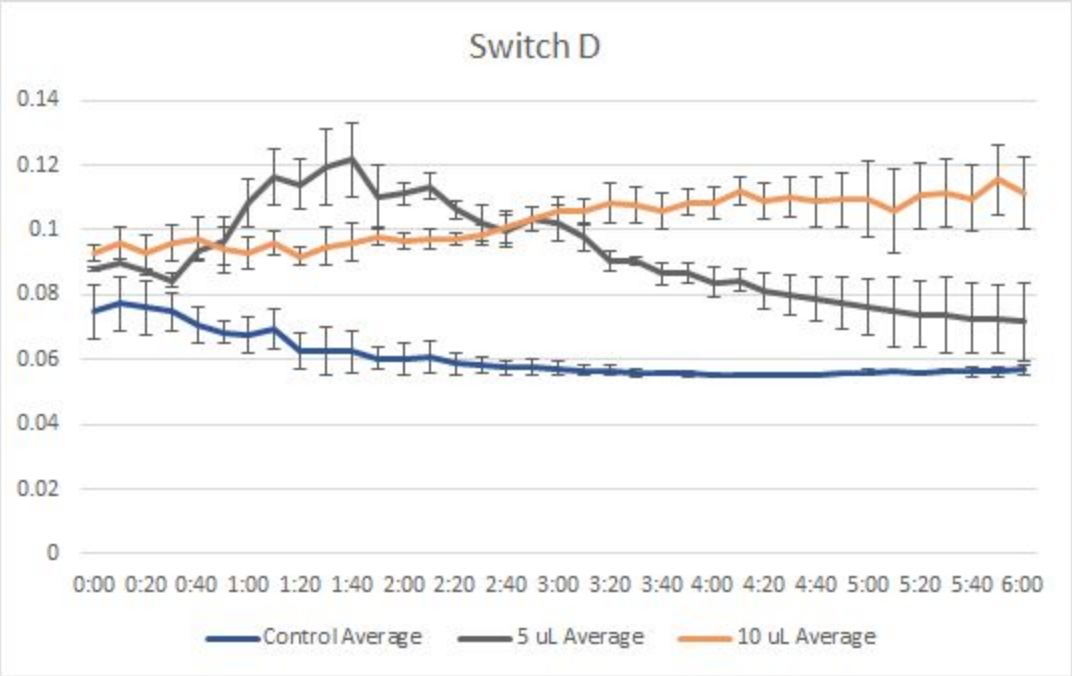
Add switches

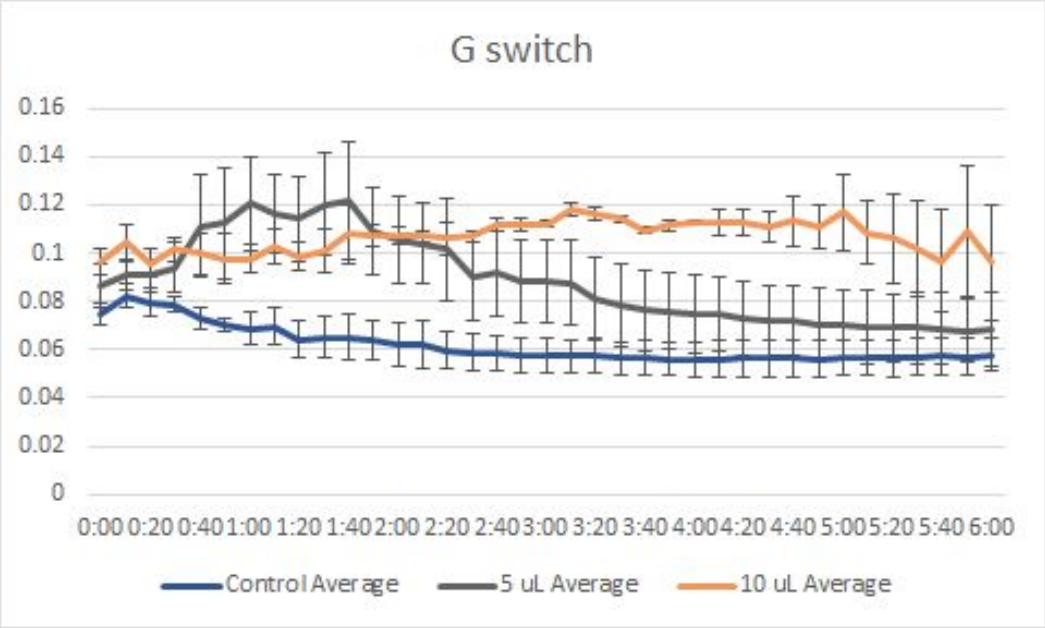
Separate out 14.46 uL for D control // 13.77 uL for G control
with 0.54 uL water // with 1.23 uL water

Add trigger to remaining

Add 5 uL to wells, dilute to appropriate volumes

6-hour time course with readings every minute, lid on





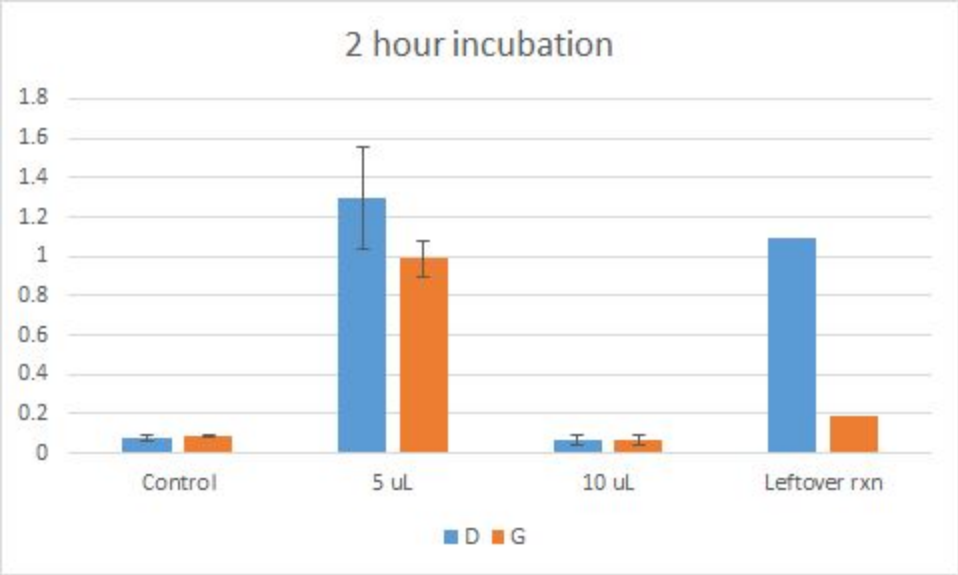
Dilute all wells to 10 uL to read final absorbances

Plate: Row K: D switch
Row L: G switch

Columns 1-3: control
Columns 4-6: 5 uL
Columns 7-9: 10 uL

Column 10: leftover reaction incubated in heat block

*taken from bottom of plate



Results quite different from last week

5 uL reactions consistently proceeded

10 uL reactions barely orange

Put plate back in plate reader for overnight time course

Anneal (Maddie/Maya)

Sequences received

Was not LacZ like we believed

Found another file with LacZ in it, will transform that

Gel Extraction of RBS-T3 and Plasmid Backbone

Digest

5 ug DNA, 40 uL reaction

Cut with XbaI and PstI (2 uL each)

4 uL Fastdigest Buffer

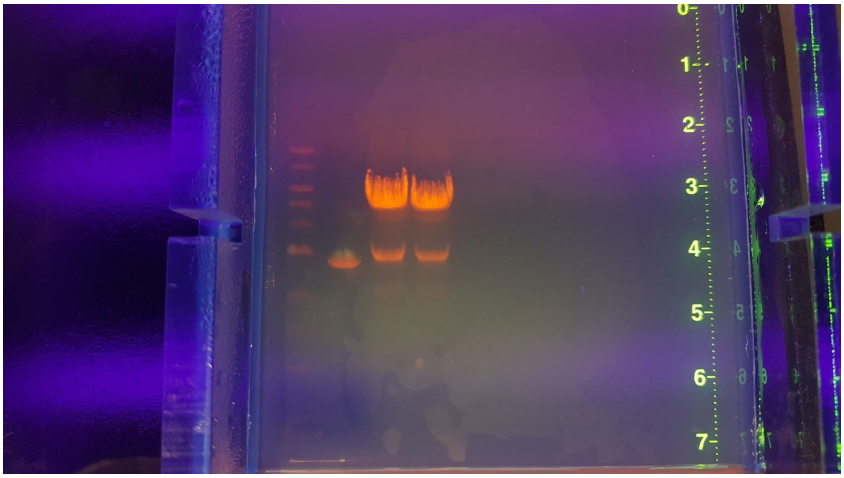
15 uL DNA (173.5 ng/uL)

8.-- uL DNA (--- ng/uL)

8.-- uL H2O

Gel Extract Digest Protocol (45 min at 37C, 20 min at 65C)

Gel



Lane 1: 1kb Ladder
Lane 2: Control
Lane 3: 20 uL Digest
Lane 4: 20 uL Digest

Gel extract

DNA concentration:

Fundraising (Claire)

Wednesday, July 27

Cell-free Collins dilution (Claire)

Overnight time course: wells dried up

10 uL reaction darker, but not fully purple

*taken from bottom of plate

Time course data is function of evaporation more so than reaction

Modeling (Aife/Maya)

Talked with Natasa about modeling toehold and population/economics of lead

Miniprep Liquid Cultures (Maddie)

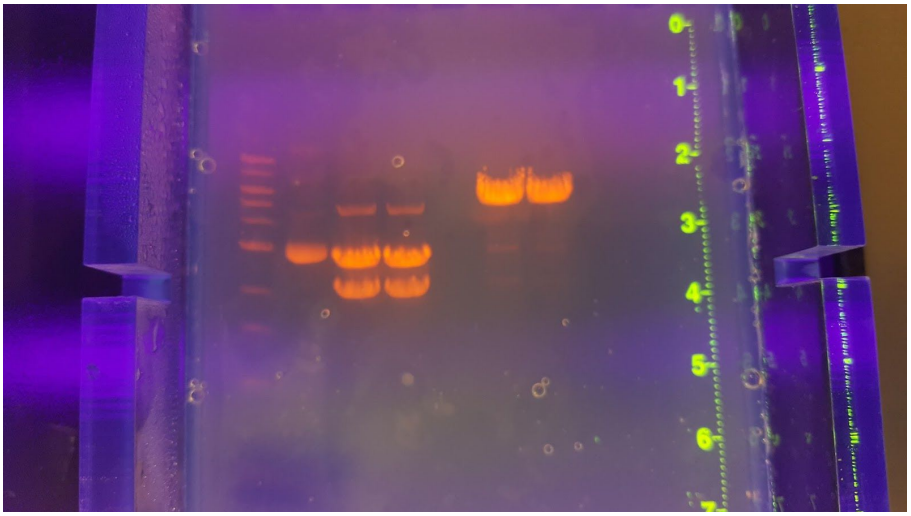
Gel Extraction of RBS-T3

Digest with EcoRI and PstI (Maddie)

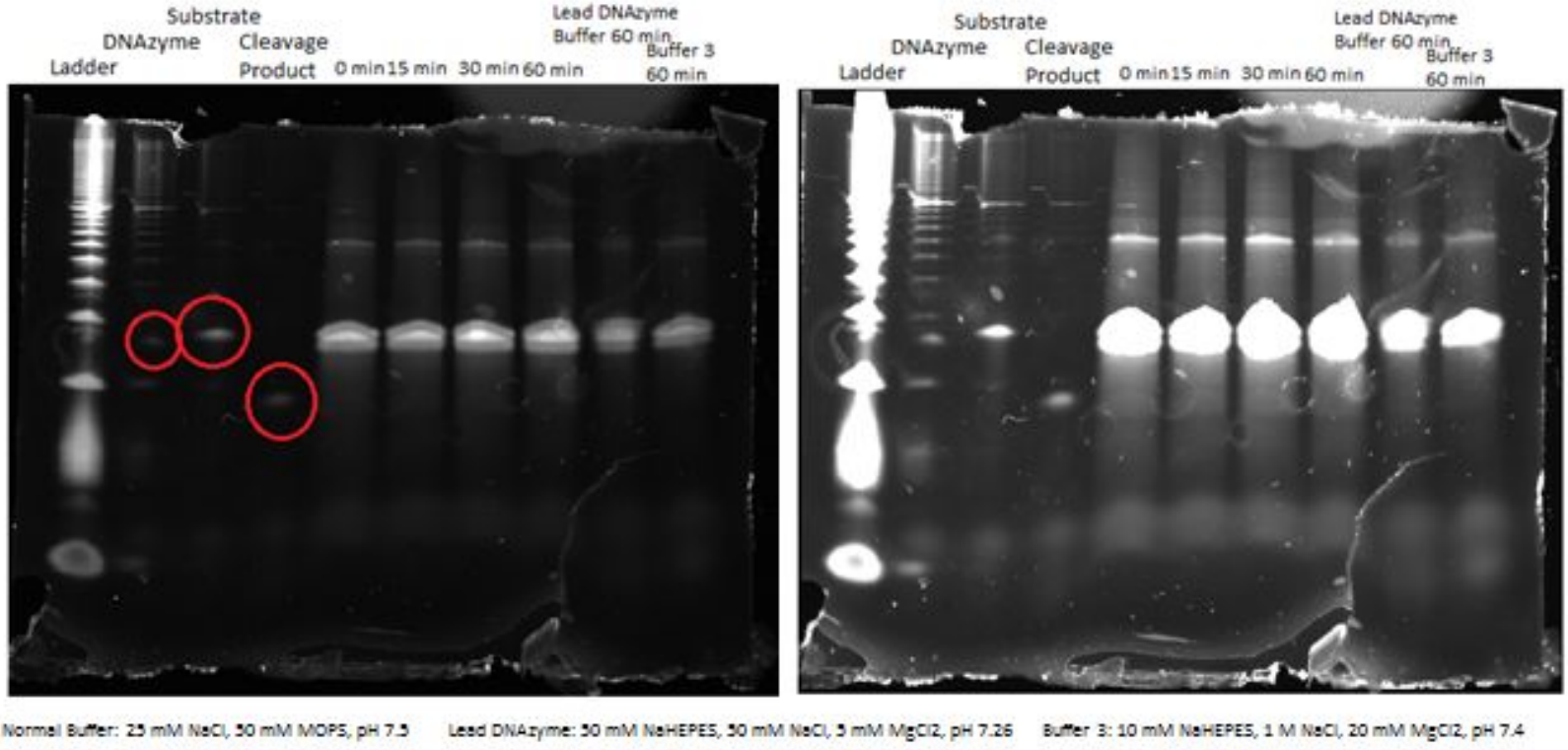
50 uL reaction

Incubate for 2 hours

Gel



Make CM plates (Maddie/Maya)
Liquid cultures (Maya)
PT7-RBS-amilCP-Term
LacZ(?)
dPAGE DNAzyme (Praneeth)



No evidence of cleavage

Thursday, July 28

Camp BioE presentation (Claire and Praneeth)

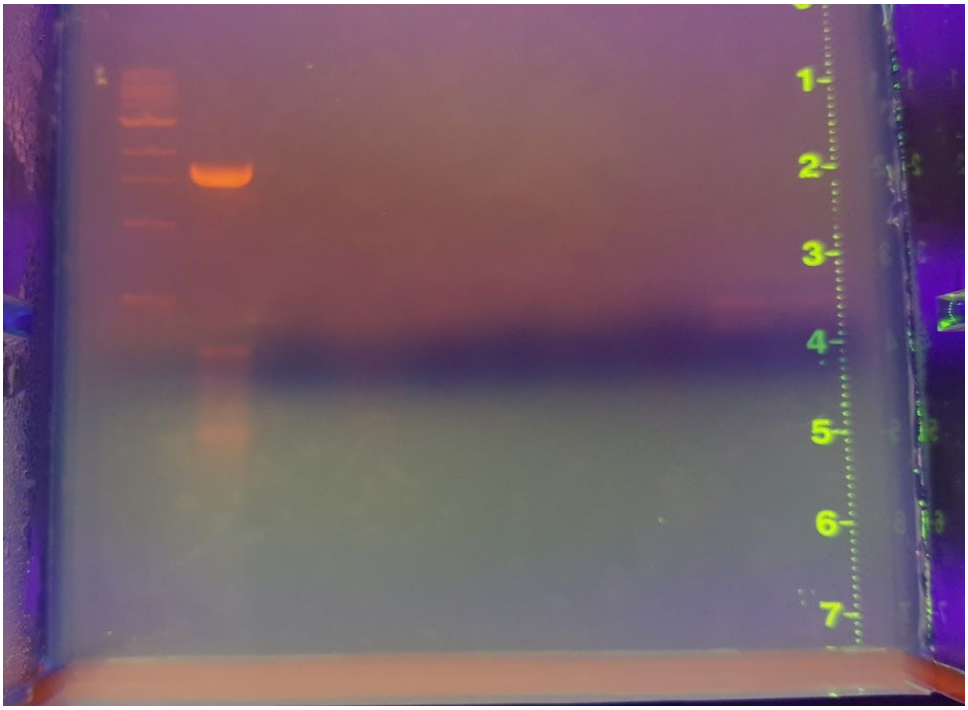
Miniprep Liquid Cultures (Maddie)
Ligation of PT3 and PT3-RBS with plasmid backbone (Maya)
- Control
- 3:1 PT3
- 7:1 PT3
- 3:1 PT3-RBS
- 7:1 PT3-RBS

PCR Amplification of T3 (Maya)
- At 55C and 52C, 20 uL reactions

Friday, July 29th

Prepare and ship LacZ and amilCP construct for sequencing (Claire)
- LA01: LacZ VR
- LA02: LacZ VF2
- LA03: amilCP VR
- LA04: amilCP VF2
- 15 uL reaction
  - 5 uL 5 uM primer
  - 500 ng plasmid = 1.26 uL amilCP // 5.44 uL lacZ
  - Water to volume = 8.74 uL // 5.44 uL

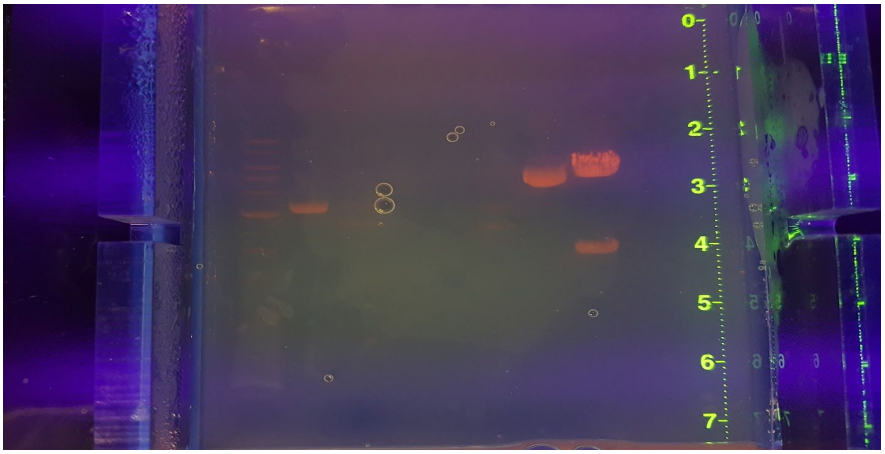
Colony PCR of PT3 and PT3-RBS (Maya)
- 100 uL Master Mix (http://2013.igem.org/Colony_PCR_Protocol)
  - 20 uL 5X Reaction Buffer
  - 15 uL VR (5 uM)
  - 15 uL VF2 (5 uM)
  - 2 uL dNTP
  - 2 uL DMSO
  - 44 uL H2O
  - 1 uL Phusion
- 10 uL per reaction
  - Used pipette tip to pick part of colony and dipped into tube
- Used BioBrick Primer cycle in PCR machine
- Gel (1.5%)
  - Lane 1: 1kb ladder
  - Lane 2: 10bp ladder
  - Lane 3: Plasmid Backbone
  - Lane 4: Colony 1
  - Lane 5: Colony 2
  - Lane 6: Colony 3
  - Lane 7: Colony 4
  - Lane 8: Colony 5
  - Lane 9: Colony 6
  - Lane 10: Colony 7



Not 100% sure what happened here:

- Best guess is that control somehow (??) got into lane 2
- Ran the gel too long
- Use imager next time (Praneeth gone so no access)

T3 amplification check along with amilCP construct check (Maddie)



PAGE check annealing with Nick (Claire)

15% gel (poured in an extra mL of TBE)

16 uL sample + 4 uL loading buffer with bromophenol blue

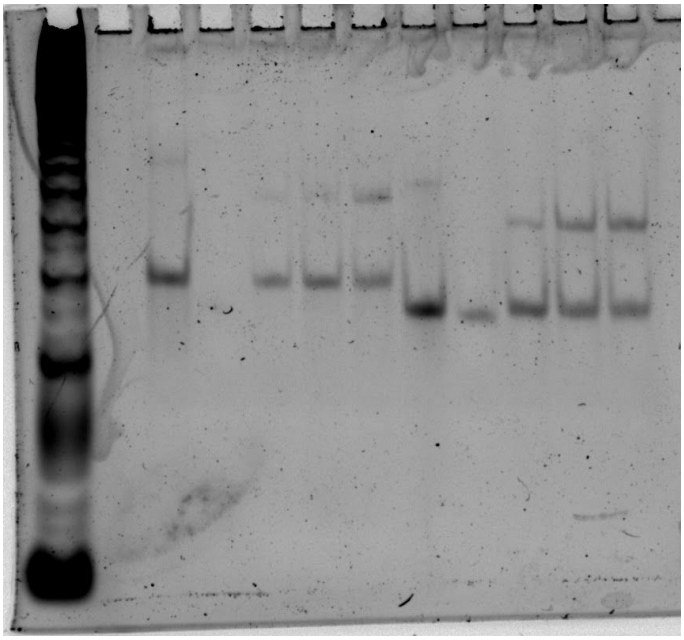
2 uL 10 bp ladder with 1 uL loading buffer

Lane	1	ladder	7	D 50 nM : 100 nM
	2	(empty)	8	G substrate, 50 nM
	3	D substrate, 50 nM	9	G catalytic, 50 nM
	4	D catalytic, 50 nM	10	G 50 nM : 50 nM

5	D 50 nM : 50 nM	11	G 50 nM : 75 nM
6	D 50 nM : 75 nM	12	G 50 nM : 100 nM

Run at 100V for 1 hour

SYBR Gold stain 20 min



Not sure what happened to D catalytic strand

Duplexes are formed (especially G 1:1.5 and G 1:2)

Excess substrate strand

Gel from 07-15 was just viewed from the bottom. Consistent with this gel

YAYYYYYY

Fundraising (Claire)